Protocol for colony PCR

Taq master mix	5ul
Forward primer	1ul
Reverse primer	1ul
dd water	2.5ul

after preparation of the reaction mix, pick a single colony and dip in the mix. The reaction condition settings are as follows:

94℃	30s
55℃	30s
72℃	1kb/min
Cylces:	30-35

Protocol for PCR with PrimeStar HS DNA polymerase

5XPrimeSTAR® Buffer	10ul
dNTP Mixture(2.5mM)	4ul
Forward primer	1ul
Reverse primer	1ul
Template	<200ng
PrimeSTAR® HS (2.5 u/ul)	0.5ul
ddwater	up to 50ul

In 50ul reation system it takes much time for the sample to change from one temperature setting to another temperature setting, which has

negatie effect to the PCR. We suggest mix the agents listed above together then divide them into five 10ul reaction system. This procedure gives better result according to our experience.